Journal of Pharmaceutical Research International

Volume 36, Issue 2, Page 12-27, 2024; Article no.JPRI.113039
ISSN: 2456-9119, NLM ID: 101716968
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Formulation Techniques, Characterization of Nanoemulsion and their Pharmaceutical Applications: A Comprehensive Technical Review

Muhammad Abid Mustafa [a*], Namra Rasheed [a++], Hamza Farooq [a++], Muntaha Fazal [a++], Emaan Asif [a++], Hamna Shafiq [a++], Muqadas Amin [a++], Gohar Jaan [a++], Amna Nadeem [a++], Tooba Farooq [a++], Neha Fatima [a++], Sadia Ahmad [a++] and Muhammad Zahid Iqbal [b]

[a] Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Lahore University of Biological and Applied Sciences, Lahore, Pakistan.
[b] Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Lahore University of Biological and Applied Sciences, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2024/v36i27498

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113039

Review Article

Received: 14/12/2023
Accepted: 19/02/2024
Published: 26/02/2024

ABSTRACT

Nanoemulsions are stable liquid-in-liquid dispersions with large surface areas, strong stability, optical transparency, and adaptable rheology. These submicron-sized emulsions are being studied for drug delivery and targeting in cosmetics, diagnostics, drug therapies, and biotechnologies. This

---

[++] Research Student;
**Corresponding author: E-mail: abidbhatti222@gmail.com;*

review explores various techniques for developing and characterizing nanoemulsions, their common formulations, merits, demerits, and current and future applications due to their unique structures and chemistries. The study emphasizes the importance of ideal formulations for nano-droplet systems, including droplet size, solubilization, colloidal stability, and optical and rheological characteristics.

Keywords: Nanoemulsion; high energy homogenization; low energy homogenization; drug delivery system; dispersion system.

1. INTRODUCTION

Nanoemulsion drug delivery systems are a promising tool for delivering and improving the bioavailability of hydrophobic drugs and bioactive food components in the blood. The majority of drugs are hydrophobic (lipophilic) in nature, thus leads to low solubility and bioavailability problems; the bioactive food components also show low bioavailability's in conventional doses [1,2,3]. In the food industry, nanoemulsions are being explored to encapsulate, stabilize, and deliver lipophilic constituents like flavors, omega-3 fatty acids, vitamins, preservatives, nutraceuticals. They have a number of potential advantages over conventional emulsions like incorporation into optically transparent products, may enhance the texture, stability, and bioavailability of products [2-5]. A widely used, high-energy method to reduce the droplet size of nanoemulsions is ultrasonication. In this method, mechanical vibrations from ultrasound waves (> 20 kHz) create sinusoidal pressure variation in the emulsion system [6,7,8]. The objective of this review is to explore various techniques for developing and characterizing nanoemulsions, their formation and stability theories, and their current and future applications due to their unique structures and chemistries.

2. FORMULATION TECHNIQUES OF NANOEMULSION

2.1 High Energy Methods

i. High-pressure homogenization

Nano emulsions are often produced in high-pressure homogenizers.

Construction: A pump used in high-pressure homogenizers increases the dispersion's pressure by 500–5000 psi.

The homogenizing valve's opening through which fluids are forced [1].

Working Principle and Mechanism: A coarse emulsion is formed using a high shear mixer and introduced into a high-pressure homogenizer, resulting in a fine emulsion. Forces like turbulence, shear, cavitation, shock, shear stress, pressure gradient, and expansion shear cause droplet breakage [2]. The homogenizer employs various nozzle types to enhance droplet fracturing, result in a nanoemulsion with a lipophilic core separated by a monomolecular phospholipid layer [3]. The final product undergoes hydraulic shear and turbulence, forming a small particle emulsion, effectively reducing the size of coarse emulsions by mixing oil and water separately.

Operational Parameters: The droplet size decreases with increasing homogenization pressure, emulsifier adsorption rate and interfacial tension, and increases due to the decrease of these factors.

Merits: The homogenizer produces smaller particle sizes by delivering the power in the shortest possible time with the most homogeneous flow (up to 1 nm). It is a high-efficiency process.

Demerits: It is a heavy reliance on energy. An increase in emulsion temperature during processing.

ii. Ultrasonication

Ultrasonication is a high-energy method used to decrease droplet size in emulsions by causing a sinusoidal pressure shift due to mechanical vibrations above 2 kHz [5].

Construction: A piezoelectric probe is used to produce a strong disruptive force by the help of its tip.

Working principle and mechanism: The process involves mixing a homogenous oil phase into an aqueous phase, creating a coarse emulsion, and then subjecting it to ultrasonication

to create nanoemulsions. Bubbles are produced through cavitation, causing droplets to condense.

Operational parameters: Increased sonication time and input power decrease droplet size. Probe placement, depth, and contact with solid surfaces affect pressure distribution and wave reflection [6].

Merits: Less energy expenditure by using it.

Demerits: Contamination would be caused by the probe.

iii. Microfluidization

The droplet size of the previously formed coarse emulsion is reduced by using microfluidizers [8]. The working mechanism for size reduction includes hydraulic shear, impact, attrition, impingement, intense turbulence and cavitation [6].

Construction: A microfluidizer consists of an inlet feed, a pressure intensifier pump, an interaction chamber, a cooling coil, and an outlet.

Working principle and mechanism: The pre-emulsion feed is divided into two channels using a stainless-steel block, which are positioned inside the device. The channel of the block gets smaller until it is about 75 µm wide. The two feeds are made to collide directly. As a result, a very high shear is generated, resulting in the production of nanoemulsion [8].

Operational parameters: The energy input can be increased by adjusting the operating pressure or emulsification time, which can be achieved by repeating the procedure multiple times [8].

Merits: There is no contamination of the feed material [6]. Continuous mass production of large quantities of goods and preparations is possible [10].

Demerits: As the emulsification begins quickly, the biopolymers used in this method are unable to stabilize recently disrupted droplets in that short amount of time due to the high energy density that is produced during the procedure. In "over-processing," the effects of droplet disruption are amplified by recoalescence, leading to an increase in EDS (energy density spread) when the energy density is raised above the optimum level [8].

2.2 Low energy methods

Low energy methods use internal physical properties of the system such as temperature or composition to produce nanoemulsions [11].

i. Phase inversion by temperature

The process involves heating surfactant, oil, and water, stirring until cool, to produce nanoemulsions with small droplets and narrow particle sizes, ensuring high reliability and consistency [11].

Working principle and mechanism: The PIT method uses temperature fluctuations to alter the hydration characteristics of nonionic surfactant head groups. At low temperatures, the head-group is highly hydrated, while at high temperatures, it becomes dehydrated. This affects the surfactant's water solubility and ideal surfactant monolayer curvature [11].

Phase inversion temperature determination (PIT): The hydrophilic-lipophilic balancing temperature (HLB) was calculated using electrical conductivity method. Mixtures of decane, water, and surfactant were agitated, and the HLB temperature, or phase inversion temperature, was determined by heating [12].

Phase inversion by temperature-driven emulsification: There are two steps involved. Initially, a temperature up to 15 C higher than the PIT corresponding to the given surfactant concentration was achieved by simultaneously and separately heating the water phase and the oil phase containing the surfactant. When the oil phase reached that temperature, water was added, and the mixture was removed from the heat source and allowed to cool naturally before being transported to the PIT. The oil phase was solubilized into a bicontinuous microemulsion at the HLB temperature, then chilled to 25 degrees Celsius and continuously stirred during chilling [12].

Process variables: Surfactant concentrations affect the PIT, droplet size, particle morphology, and storage stability. The oil phase composition affects the turbidity, which has a direct impact on the PIT [11].

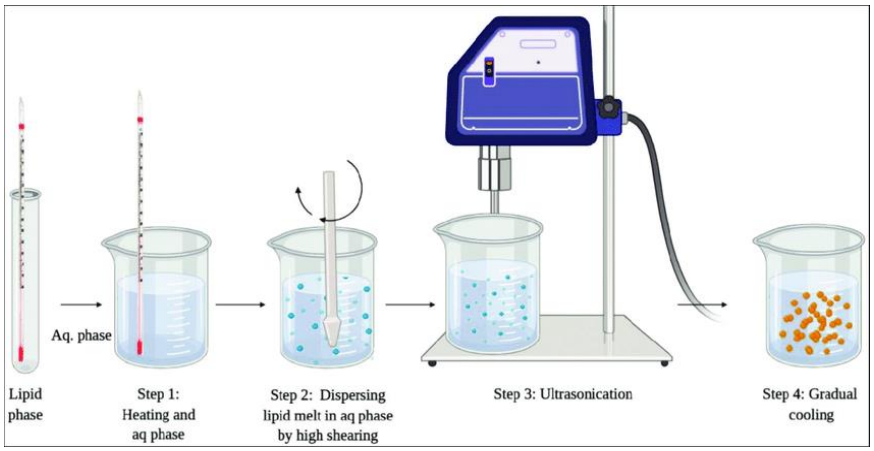

Fig. 1. Formulation of nanoemulsion using cold high-pressure homogenization technique [4]

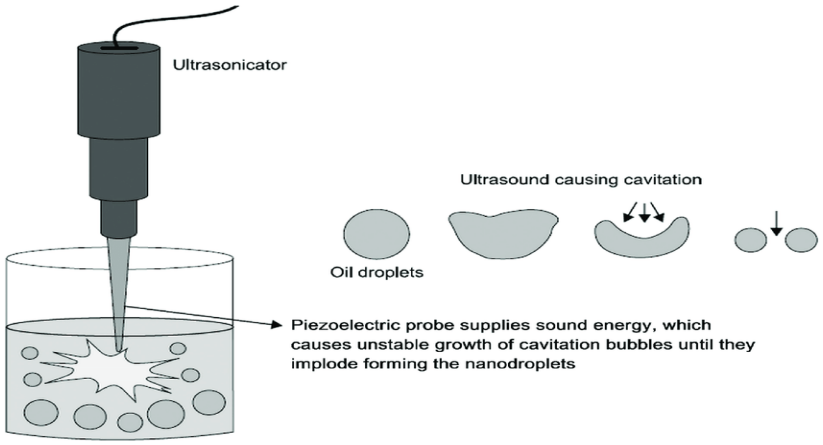

Fig. 2. Ultrasonication technique [7]

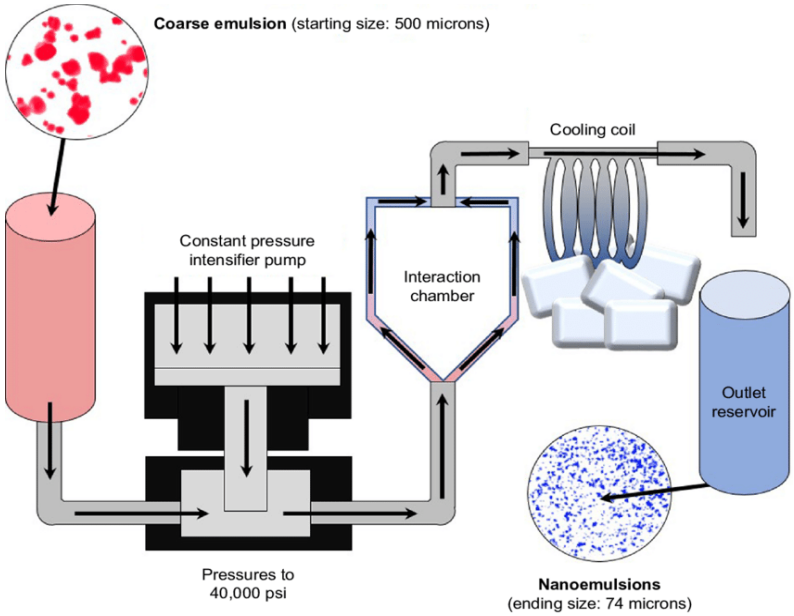

Fig. 3. Microfluidization technique [9]

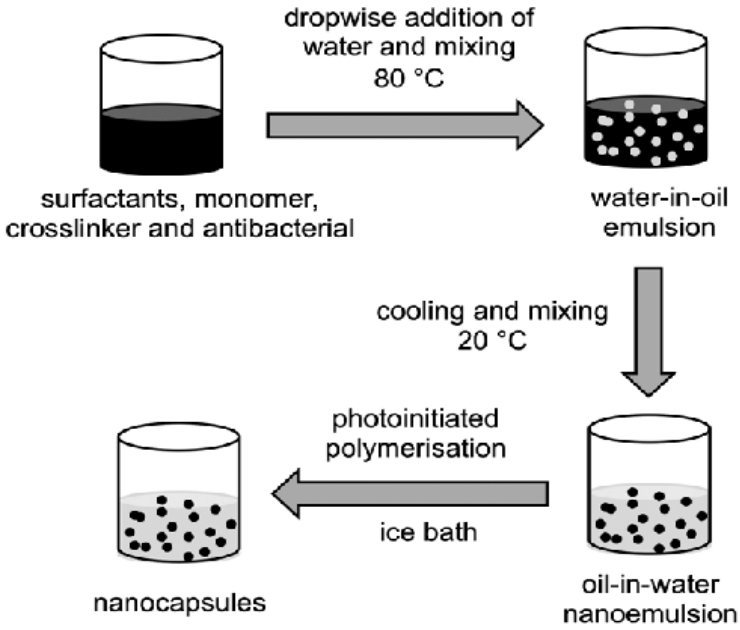

Fig. 4. Mechanism of nanoemulsion formation by PIT method [11]

Merits: It is simple to perform and implement. There is no need for sophisticated equipment.

Demerits: Higher surfactant concentrations cause instability problems.

ii. Solvent displacement method

This method involves combining the organic phase—which contains the oil dispersed in a solvent such as ethanol or acetone—with the aqueous phase, which contains the surfactants [13].

Working principle and mechanism: Emulsification occurs spontaneously due to diffusion of organic solvent, which may be removed later by vacuum evaporation [13].

The solvent displacement method precipitates polymers from an organic solution, dissolves them in a semipolar solvent, and then adds them to an aqueous solution containing stabilizer, forming nanoparticles instantly [14].

Process variables: The volume, viscosity, type of surfactant, temperature, and droplet size all play a role in the selection of the emulsifying device. The optimal nanoemulsions are achieved by the optimization of setup parameters [13].

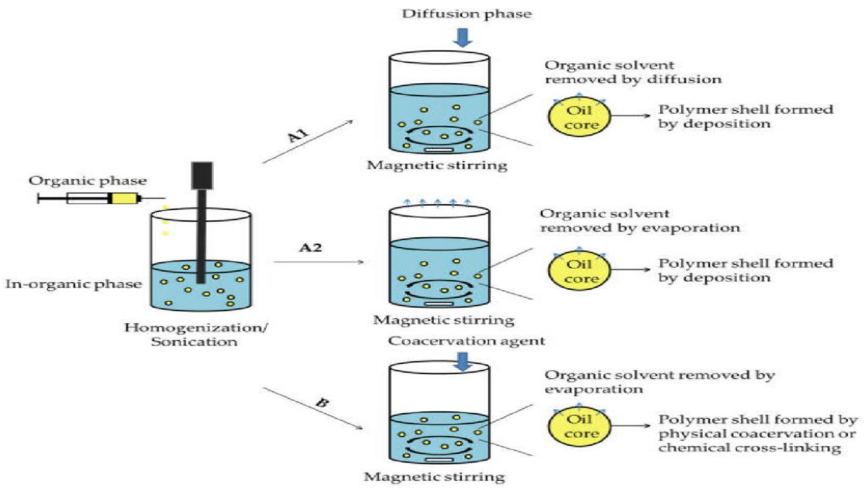

Fig. 5. Representation of solvent displacement method [15]

Merits: There is no heating required for it, and there is no requirement for an organic solvent in it.

Demerits: The need for a large ratio of solvent and oil for the production of small droplets in the disperse phase [13].

iii. Self-Emulsifying Nanoemulsion (SNEDDS)

SNEDDS are isotropic mixtures of oil, surfactant, co-surfactant, and drug that form an aqueous oil-in-water emulsion with little stirring.

Working principle and mechanism: SEFs are mixtures of oil, surfactant, co-surfactant, and co-solvents that form a transparent, isotropic solution that emulsifies under gentle agitation and is similar to the gastrointestinal tract. (GIT).

Process variables: There are many factors that affect SNEDDS. High-dose drugs are not suitable for SNEDDS due to their limited water solubility and difficulty in delivering lipids. SNEDDS' solubility in the oily phase is crucial, and larger surfactant or co-surfactant roles can lead to precipitation.

Merits: It would increase consistency in drug absorption. Drugs are selectively targeted towards a specific absorption window in the GI tract. Drugs are shielded from the gut environment. Variability in the process has been reduced, including food effects. It would increase oral bioavailability, allow for dose reduction, and have high drug loading efficiency. The cost of production will be low.

Demerits: There are no good predictive in vitro models for evaluating formulations. Traditional dissolution methods are ineffective because formulations are not dependent on digestion prior to drug release. Further development and validation of the in vitro model is required. There are various lipid-based prototype formulations that must be developed and tested in vivo. The GIT system may be irritated by chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30–60%). The dilution effect of the hydrophilic solvent may increase the precipitation tendency of the drug. Validation of multi-component formulations becomes more difficult [17].

iv. A Review on Self Emulsifying Nanoemulsion

BCS Class IV Drugs: Class IV drugs pose challenges in oral formulations due to low solubility and permeability. Self-emulsifying drug delivery systems (SEDDS) can increase solubility and permeability by increasing drug concentration, inhibiting enzymatic breakdown, and promoting intestinal lymphatic transport [18].

3. COMMONLY PREPARED NANOEMULSIONS BY DIFFERENT FORMULATION TECHNIQUES

3.1 Isoflurane-Loaded Nanoemulsion Prepared by High-Pressure Homogenization

The process involved preparing medium-chain triglycerides in Lipoid S75® and adding Isoflurane before forming an emulsion. The aqueous phase involved mixing water, sorbitol, and polysorbate 80 at 30°C with magnetic stirring. The oily and aqueous phases were emulsified using a high-shear mixer, producing a coarse emulsion. The emulsion was further treated using a high-pressure homogenizer at 10°C using the output cooler. The process involved separate preparation steps for each stage [20].

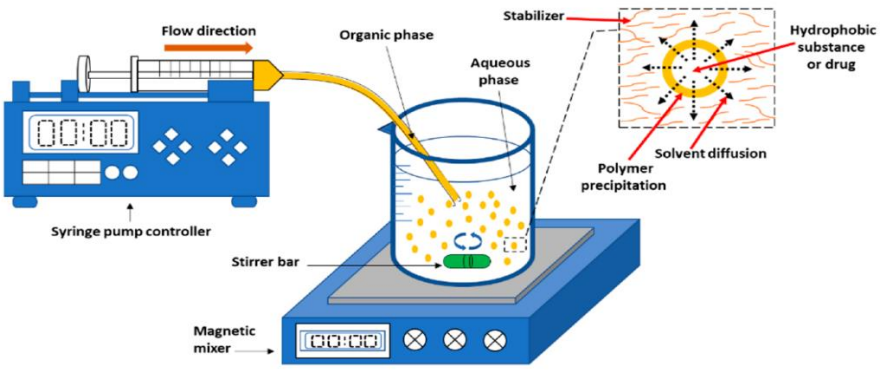

Fig. 6. The nanoprecipitation process is illustrated in a diagram [16]

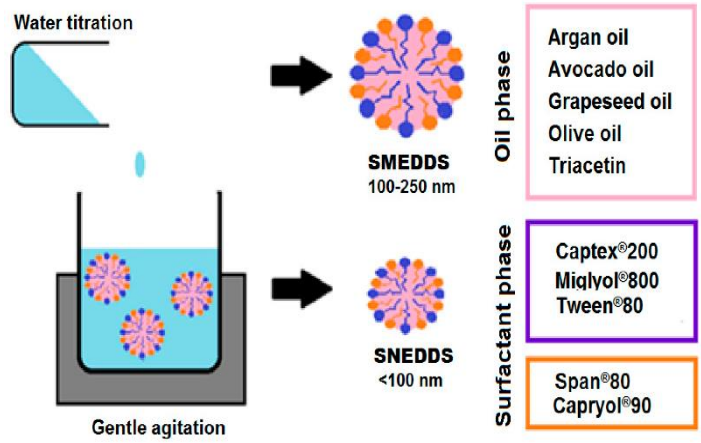

Fig. 7. Self-Emulsifying Method [19]

3.2 Preparation of a Novel Curcumin Nanoemulsion by Ultrasonication

Curcumin Nanoemulsion (Cur-NE) was developed using high-energy ultrasonication, oil, surfactants, and Milli-Q water. The final nanoemulsion was produced with 40.0% ultrasonication intensity, 10.0 minutes of ultrasonication time, and 50°C temperature. Cavitation facilitated the process, resulting in improved Brownian motion and smaller globule sizes for extended storage [21].

3.3 Production of Sub-Micron Emulsions by Ultrasound and Microfluidization Techniques

The study involved preparing coarse emulsions and passing them through an air-driven microfluidizer. The pre-emulsion was fed through a glass reservoir and divided into two channels in a ceramic interaction chamber. The jets of pre-emulsion collide head-on at high pressure, causing extreme shear. The typical pressure of liquid jets flowing through the channels is 120 MPa due to mechanical amplification of 232. The emulsions' volume flow rate was 4 × 10^-6 m3/s 60 MPa for one cycle. The experiments were duplicated [8].

3.4 Preparation of Cinnamon Nanoemulsion by Phase Inversion by Temperature Method

The study involved blending cinnamon oil and Medium-chain triglycerides, heating each solution to 15°C, determining the PIT for minimal turbidity, and cooling twice. The initial cooling may result in a stable microemulsion, but the system was rapidly cooled by adding 250 g of cold, deionized water and stirring continuously for three minutes. The aim was to determine the mean droplet diameter, stability, and particle size distribution of each sample by rapidly cooling it to the PIT temperature. The overall amounts of oil phase, surfactant, and water phase remained constant [11].

3.5 Preparation of Maltodextrin-Stabilized A-Tocopherol Nanoemulsions using the Solvent-Displacement Technique

The study developed a-tocopherol nanoemulsions using solvent-displacement and different ratios of Polysorbate 20 and maltodextrin. A-tocopherol was dissolved in acetone to prepare the organic phase, with a 1:1 volume ratio. The organic phase and Polysorbate 20 solution were added to the maltodextrin solution, homogenized using magnetic stirring. Acetone was extracted from the system using a rotary evaporator set to 30 degrees Celsius for 20 minutes [22].

3.6 Preparation of Chlorpromazine Nanoemulsion by Self-Emulsification Method

The solubility of chlorpromazine in oils, surfactants, and ethanol was studied to create SNEDDS. Formulations were created by adding glycerides and surfactants to a 20 mg dose, heating, and adding ethanol. After 48 hours, formulations were checked for phase separation, turbidity, and particle size characterization [23].

4. CHARACTERIZATION OF NANOEMULSIONS

4.1 Droplet Size

4.1.1 Transmission electron microscopy

Introduction: To investigate the form and size of nanoparticles, transmission electron microscopy is used. For this, we use a 300-mesh copper or carbon transmission electron microscopy grid with a glow discharge. Samples are prepared by incorporating dilute solutions and then drying at room temperature. For transmission electron microscopy, only the most stable emulsions can be used, [24].

Method: The specimens were positioned on a polycarbonate base, and any surplus water was allowed to evaporate naturally at room temperature (25 ± 8°C). They are then dried using carbon dioxide in a critical point dryer before being sputter-coated in gold using a metallizer. Finally, the samples were scrutinized using a scanning electron microscope with an operational accelerating voltage of 20 kV [25].

4.2 Atomic Force Microscopy

Introduction: Due to its simplicity and resemblance to real nanoemulsion systems, atomic force microscopy is the preferred method for evaluating the interfacial properties of nanoemulsions. This technique is to examine the physical attributes of nanoemulsified coatings, such as average roughness, root mean square roughness, surface morphology, and droplet size of nanoemulsions [26].

Method: It is essential to dilute most nanoemulsions with distilled water between 100 and 1000 times in order to prevent droplet aggregation and coalescence. Subsequently, the diluted solution is spread over the previously split mica substrate. Before dehydrating, the accumulated droplets are sometimes cleaned with distilled water and kept at room temperature for a whole night in a dust-free environment. Alternatively, the drying process can be accelerated by employing a furnace or heater. Adsorption occurs when charges on a sample and mica surface are attracted due to droplet binding on the substrate surface. The size and shape of the droplets were then determined using the images taken [26] and then observed by atomic force microscopy with a resolution of up to 0.1 nm [24].

4.3 Interfacial Tension

Introduction: Interfacial tension is used to study the formulation and characteristics of nanoemulsions. Ultralow levels of interfacial tension are indicated by phase behavior when the surfactant phase is in equilibrium with the oil and aqueous phases. Spinning drop equipment may be used to measure extremely low interfacial tension values [27].

Method: Photon correlation spectroscopy is used for measuring the size of nanoemulsion droplets. It is done by using a volumetric flask in which 0.1 mL of formulation and 50 mL of water are added and mixed by gently inverting the flask. Measurements are taken by setting the zeta sizer and light scattering monitor at 25 °C at a specific angle (90 or 180) [28].

4.4 Zeta Potential

Introduction: Zeta potential is a measure of particle charge, which is an important characteristic to determine the stability of nanoemulsions. High zeta potentials indicate stability, which means solutions show resistance to aggregation. Low potential indicates attraction exceeds repulsion, and dispersion flocculates. This measure indicates forces between particles at the nanoemulsion surface, which helps in the stabilization of nanoemulsions. For electrostatically stable emulsions, the zeta potential must be 30 mV. For nanoscaled particles, zeta potential is influenced by factors such as particle source, electrolyte concentration, pH, hydration, and particle morphology [24].

Method: The Zeta sizer Nano ZS Apparatus is used for the determination of Zeta potential by using the electrophoretic mobility of particles in an electric field. The zeta potential of the formulation was measured using a Beckman Coulter Delsa Nano C Particle Analyzer, USA. Zeta's potential value, which is determined by measuring the electrostatic charge and the attraction or repulsion between the particles, indicates the stability of the nanoemulsion. To maintain stability, an emulsion has to attain a minimum of 30 mV (positive or negative) of zeta-potential value [29].

4.5 Polydispersity Index and Particle Size

A spectrophotometer is employed for this purpose to determine the consistency of the

droplet size of nanoemulsions. It represents the standard deviation of the mean droplet size ratio. Decreased consistency will increase polydispersity [27].

Method: In order to circumvent the effects of multiple scattering, the measurement of polydispersity index and z-averages (mean particle size) was performed using ultrapure water (1:10) and a Zeta sizer Nano-ZS (Malvern Instruments Ltd.) [30]. Prior to measurement, the samples were diluted, and 25°C and 1.33 were selected as the temperature and water's respective refractive indices. The averages and standard deviations of the three measures were noted. The polydispersity index is used to describe the size of droplets and their distribution width. A value between 0.1 and 0.25 indicates a restricted size distribution, whereas a value greater than 0.5 indicates a broad range of particle sizes [29,31].

4.6 Refractive Index

Introduction: The refractive index is the net value of the components of a nanoemulsion and indicates the isotropic nature of the formulation. It is the technique for assessing whether the formulation is transparent or not, as well as the thermodynamic stability analysis of the sample [27].

Method: It is determined by putting a sample drop on a slide and then comparing it with water with a refractive index of 1.33 using a refractometer. If the comparison of the system's refractive index is relatable to the water's refractive index, then the formulation is transparent. The refractive index was determined by using a refractometer [27].

4.7 Conductance

Introduction: A conductometer is used to determine the conductance of the sample, i.e., nanoemulsions. An electrical conductivity tester with an 11+ USA conductance meter was used to test the electrical conductance of the nanoemulsion at 25 degrees. Three runs of this test were conducted to ensure uniformity [32].

Method: In this method, electrodes are immersed in an emulsion system that is supplied with an electric source and a lamp. If there is an o/w-type emulsion, then water conducts current and the lamp lights up due to the flow of current between the electrodes. If oil is in the exterior phase, the lamp is dark because emulsion is absent [27].

4.8 Viscosity

Introduction: It is a key characteristic of nanoemulsions. The resistance to flow of fluids is termed viscosity, or the friction that exists within fluids. The most frequently employed instrument to measure viscosity is the Brookfield viscometer.

Method: The viscosity of the produced nanoemulsion was measured using the Brookfield DV-II+ Pro viscometer at 25°C without dilution by taking the average of three data points at a specific shear rate. After the mixture had been in the beaker for five minutes, spindle readings were taken at 0.5, 1, 2.5, and 5 rpm at the maintained temperature and at room temperature for 12 weeks, which suggests that the lower the storage temperature, the greater the viscosity of nanoemulsions. The viscometer's accompanying dial was read and recorded. According to the power law model, emulsions exhibit shear-thinning behavior under shear rates. It gives three ranges for n (flow behavior index): $n<1$ for shear-thinning fluids, $n=1$ for Newtonian fluids, and $n>1$ for shear-thinning fluids. Emulsions show less than 1 n value [32].

$$\text{Viscosity} = \text{Mass}/\text{Volume}$$

The viscosity of nanoemulsions can be determined at various shear speeds [27].

4.9 Dye Test

Microscopic analysis is done for clear understanding. If the emulsion is o/w, then it continuously absorbs water-soluble dye. Conversely, without emulsion, it only uptakes water-soluble dye in the dispersion phase, and the color is not uniform [27].

4.10 Creaming Test

Following homogenization, 10 milliliters of the nanoemulsions were immediately put into a test tube, firmly sealed, and kept at room temperature (25 ± 2°C) for seven days.

The creaming index was determined after a visual inspection to evaluate the cream's stability.

$$\text{Creaming index percent} = (HL/HE) \times 100\%$$

Where HL is the entire height of the cream layer and HE is the overall height of the emulsions [33]. An increased creaming index indicates the presence of emulsion instability, which can be attributed to flocculation, aggregation, coalescence, or high particle size [29].

4.11 Creaming Stability

Visual observation was used to identify the creaming, which was then quantified using the creaming
index.

> Creaming index percent = (cream layer height/total emulsions height) × 100 [29]

4.12 Melting Resistance

To evaluate the melting behavior, nanoemulsion containers were taken out of the freezer at a temperature of -20°C in order to measure the meltdown point. The samples were placed on rectangular stainless-steel wire mesh screens measuring 1.5 by 1.5 cm by 2.5 by 2.5 mm, which were placed on top of a funnel that was attached to a graduated cylinder. The nanoemulsion was then stored in a temperature-controlled room at −25°C. Weighing and recording the water-dripping volume every 10 minutes for a maximum of 90 minutes. Both the instant that the first nanoemulsion drop dripped and the moment it completely melted were timed and recorded. A graduated cylinder was used to record the amount of un-melted nanoemulsion at 30-minute intervals [29].

4.13 pH Values and Total Soluble Solid Content

At −20°C, the total soluble solid content was measured in Brix as the result of triplicate measurements made with a Pocket Pal-1 refractometer. The pH was calculated using the pH meter by submerging the instrument bulb into 30 mL of each produced formulation [29].

4.14 Texture Analysis

The nanoemulsion samples were kept in plastic containers at -20°C for a whole day in order to conduct the texture analysis. At room temperature (25 ± 2°C), measurements of firmness, hardness, consistency, cohesiveness, and viscosity index were made using a texture analyzer TAXT2i fitted with a 2-mm-diameter acrylic cylindrical probe. The samples' geometrical centers had a penetration depth of 10 mm, and their penetration rates were 1 mm/s. After being hardened at -30°C, the nanoemulsion was sliced to fit into a tiny cylindrical cup with a diameter of 4.5 cm and a depth of 30 mm. It was then tempered overnight to -15°C in preparation for the analysis. The penetration speed of the probe was 2 mm/s up to a 20 mm distance [29].

4.15 Sensory Evaluation

The sensory analysis was performed on the nanoemulsion samples. A group of people of different age groups were selected for the sensory analysis. Using a 9-point hedonic scale—where 1 represents a very low preference and 9 denotes a very high choice—four nanoemulsion samples were evaluated for taste, preference, appearance, and consistency [29].

4.16 Percentage of Transmittance Measurement

The transmittance study was used to assess the clarity of the prepared nanoemulsions. A UV-Vis spectrophotometer was used for this investigation, with deionized water serving as the blank at the drug Lambda max of 210 nm [34].

4.17 Dynamic Light Scattering (DLS) Spectrophotometer

The DLS measurement is obtained at 90˚ with a 632 nm neon laser. The instrument determines the size and dispersion of the particles [35].

4.18 Droplet Size Measurement

Using DLS, droplet size measurements were assessed. Prior to measurements and tests, samples were not diluted and were carried out at 25°C. No photosensitizer was used during the experiments in order to reduce fluorescence interference in the signals from light scattering [36].

4.19 Creaming and Cracking

Each multiple nanoemulsion (MNE) was sampled in 30 mL and placed in a glass bottle with a screw lid (height 65 mm and inner diameter 25 mm). The container was then allowed to stand at 25 ± 2 °C. for a day before being checked for physical changes. The permanent/irreversible division or separation of the internal/dispersed

phase (where oil and water are clearly separated) at the top of the emulsion is known as cracking, which is a physical instability. The given equation was used to calculate the cream layer height (top layer) and the overall emulsion height in the event that the emulsions are divided into cream and serum layers. This allowed for the determination of the percentage of creaming.

> Creaming (%) = 100 × Height of cream layer/Total height of emulsion [35].

4.20 Entrapment Efficiency

To determine the percentage drug encapsulation efficiency, the concentration of the unentrapped drug, or free drug, in the formulation was assessed. This concerns me since it has an impact on the medication molecule's release characteristics. After the entrapped drug was separated from the nanoemulsion formulation, an equation was used to determine the quantity of drug encapsulated per unit weight of formulation.

> % Entrapment efficiency = (amount of drug added − free (unentrapped) drug)/(amount of drug added) × 100 [35].

4.21 Thermodynamic Stability

Under thermodynamic stability tests, the problem of metastable formulation was resolved. The determination of thermodynamic stability involves three steps:

4.21.1 Centrifugation

The nanoemulsions are subjected to centrifugation at 5000 rpm for 30 minutes. The stable preparations do not show any signs of phase separation. Phase separation is observed by centrifuging the sample to determine if it is an unstable or metastable emulsion [36].

4.21.2 Heating cooling cycle

The sample to be assessed is stored at such temperatures for a duration of not less than 48 hours and is also exposed to temperature variation between 4° and 45°. Six cycles of temperature exposure are given to each formulation, which is assessed for stability [36].

4.21.3 Freeze thaw cycle

Formulation is stored over three freeze-thaw cycles between 21°and +25° minimum for 48 hours, and then its stability is assessed. Such nanoemulsions are stored in a deep freezer (-20°C) for 24 hours. Then it is removed from the freezer and kept at room temperature. The stable nanoemulsions return to their original form in 2–3 minutes [36].

4.22 Differential Scanning Calorimetry

It is a thermoanalytical technique that measures the difference in amount of heat needed to increase the temperature of the sample and reference. Both the reference and sample are maintained at the same temperature throughout the experiment. The sample should have a well-defined heat capacity over a range of temperatures. This technique is employed to detect phase transitions as a result of the melting of crystalline agents and analyze the proportion of solid fat or ice crystals in the emulsion. It is also used to detect the crystallization temperature of a mixture of surfactants [24].

4.23 FTIR

It is based on infrared radiation that is absorbed by the sample. It gives a spectrum that represents molecular absorption and transmission, forming a molecular fingerprint of the sample. This fingerprint represents characteristic absorption peaks corresponding to frequencies of vibration between atoms of material. The size of the peaks in the spectrum is a direct indication of the amount of material in the sample. The advantage of FTIR is that it determines the amount of component in a mixture as well as the quality and consistency of the sample. It gives accurate and reproducible measurements [24].

4.24 NMR (Nuclear Magnet Resonance)

This analytical tool studies compounds in liquid or solid states, providing molecular structure information, stereochemistry, and repeating distances up to 150nm. It's nondestructive, requires minimal sample preparation, and offers complementary techniques [24,37].

4.25 In Vitro Dissolution Profile

4.25.1 USP Apparatus type-II

A dissolution apparatus type II was used for a drug release investigation. 900 milliliters of pH-1.2 simulated gastric fluid served as the dissolving medium. Every nanoemulsion

formulation has gone through this investigation at various pH levels by being placed in a dialysis membrane bag and replaced with fresh medium. At certain intervals, a 5 mL sample was taken and replaced with new media. Each sample was examined to determine drug concentration using a UV-Vis spectrophotometer set to analyze at a lambda max of 210nm after being filtered with a syringe filter of 0.45 µm. Then samples are analyzed in HPLC or by other methods for determining release behavior, which is then compared with standards [34].

4.25.2 Franz cell apparatus method

The Franz cell device with a diffusion area of 1.79 cm2 and a receiver chamber volume of 16 ml was used for in vitro drug release. A synthetic cellulose acetate membrane (Merck, Brazil) was used, which had been previously moistened. The membrane was then used to separate the donor and recipient compartments. To maintain sink conditions, the receiver chamber was filled with a physiological solution containing 1% cyclodextrin as a solubilizer. Peltier-type temperature control equipment was used to keep the receptor compartment at 32 °C by employing an external thermal bath. Throughout the experiment, continuous stirring was maintained. Bubbles were avoided by sonicating the receiver solution before the experiment.

On top of the membrane, the equivalent of 10 mg of formulation was deposited. The donor compartment was secured to the receiving compartment, which was then sealed with Parafilm®. Aliquots of 500 l were collected after 30 minutes, 1, 2, 3, 4, 6, 7, 8, and 24 hours. An equivalent volume of new medium was poured to maintain the washbasin condition. A validated HPLC method was used to determine the concentration of MS in each sample [35].

4.25.3 Membrane diffusion method

Drug release studies were carried out at a temperature of 32°C using standard regenerated cellulose and Spectra. For the experiment of release, take 5g of formulation along with the receptor solution filled in the dialysis chamber for 24 hours. By using UV-visible spectroscopy, the concentration of drug in the receptor solution was analyzed by wavelength at 350nm. Surfactant was added to the solution to increase its solubility in the receptor solution. The receptor solution consists of 1.5% w/w polysorbate buffer at pH 7.4 [38].

4.26 Drug Release Kinetics

Using the Microsoft Excel® add-in DDSolver, the dissolving data were fitted to different kinetics models, including Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Hopfenberg, Peppas-Sahlin, Weibull, and Makoid-Banakar [33]. The best appropriate mathematical model for each formulation was interpreted by analyzing the adjusted coefficient R2 (Rsqr_adj) and the residual standard deviation (MSE_root) [39].

Release kinetic studies were performed to determine the efficacy of active substances. There are a number of kinetic models that describe the release of drugs from the above models [38].

4.26.1 Zero order model

Drug concentration and release rate are independent of one another in zero-order release kinetics, which describes continuous drug release from the delivery device. The following equation can be used to describe the zero-order release kinetics.

$$Qt = Q0 + K0 \cdot t \quad (1)$$

Qo = first drug released in device; Q = constant (zero order release); and Q = drug released quantity in time t. If zero-order release kinetics are being examined, the cumulative drug release (Q) vs. time (t) plot forms a straight line with an intercept at Qo and a slope of Ko [40].

4.26.2 First order model

This release kinetics model describes drug release from a system where drug concentration depends
on the release rate. The following equation can be used to characterize the medication release pattern:

$$dC/dt = -K1C$$

Another way to define it is LogC=logCo-K1t/2.303, where C is the quantity of medication released in t time, Co is the amount of drug released initially in the device, and K1 is the constant (first-order release). If a first-order drug release pattern is seen, the log of cumulative drug remaining versus time t plot forms a straight line with an intercept of log Co at t = 0 and a slope of K1/2.303 [40]

4.26.3 Higuchi model

Higuchi devised this model in 1961 and 1963 for the investigation of drug release from intractable matrices using Fickian diffusion [40]. This approach was also suggested for the investigation of medication release from low- and water-soluble substances [41]. The Higuchi model uses the square root of a time-dependent mechanism using a solid dosage form to explain drug release. The following formula can be used to describe the Higuchi model.

$$Mt/M\infty = KH * t½$$

The cumulative drug release quantity in time t and infinite time is represented by Mt and M∞, respectively. KH stands for Higuchi constant in drug release. Following the Higuchi model for drug release will result in a straight line with a slope of KH on the Mt/M∞ vs. time t plot. The diffusion process defines drug release, and the Higuchi model is based on Fick's law of diffusion. The Higuchi model was originally developed for planar systems, but it was later expanded to include a variety of porous and geometric systems [42].

4.26.4 Korsemeyer peppas model

This model explains the medication's fractional release as a function of time. It accurately describes the release of drugs from polymeric systems and is described by the following equation:

$$Mt/M\infty = Kt\hat{}n \quad \text{or} \quad Log\ (Mt/M\infty) = \log K + n\ \log\ t$$

Where K is the rate constant, n is the exponent of diffusion, which defines the drug release mechanism, and Mt/M∞ describes the proportion of drug release in time t. Plotting log (Mt/M∞), or log cumulative drug release percentage vs. log time, will show a straight line with a slope of n if this model is followed [38,40].

5. APPLICATIONS

5.1 Nanoemulsions in Drug Delivery

Nanoemulsions are utilized in various drug delivery methods, including topical, ocular, intravenous, intramuscular, internasal, and oral delivery. They utilize their lipophilic nature to dissolve water-insoluble drugs and their tunable charge and rheology to create aqueous solutions. Nanoemulsions also offer advantages for hydrophobic drugs and are used as ultrasound imaging agents [43].

5.2 Oral Delivery

Lipids can be used as nanoemulsions to increase drug absorption in the GIT, particularly protein drugs, by loading them inside lipids, thereby enhancing the overall absorption process [44].

5.3 Topical Delivery

Enhancing the permeation of drugs for topical application is challenging due to poor dispersibility and skin irritant effects. Nanoemulsions, such as soybean lecithin, tween, and poloxamer, offer a combination of penetration enhancement and concentration gradient [44].

5.4 Intravenous Delivery

Parenteral nanoemulsions deliver drugs with lower bioavailability and narrow therapeutic indices, converting into stealth nanoemulsions by coating or attaching a hydrophilic moiety, enhancing permeability and retention for tumor targeting [44].

5.5 In Cosmetics

Newer materials (nanoemulsions) are increasingly important for controlled cosmetic delivery and optimized dispersion of active ingredients in skin layers. They are suitable for transporting lipophilic compounds and supporting skin penetration, increasing active ingredient concentration. Nanoemulsions also have bioactive effects, reducing trans-epidermal water loss and strengthening skin barrier function. They are acceptable in cosmetics due to their lack of creaming, sedimentation, flocculation, and coalescence [21]. TRI-K Industries and Kemira have developed a new nano-based gel, Kemira NanoGel, to improve the effectiveness of skincare products. This technology is particularly useful in sun care, moisturizing, and anti-aging creams and provides a good skin feel [44].

5.6 Nanoemulsion in Cell Culture Technology

Cell cultures are used for in vitro assays and producing biological compounds. Oil-soluble substances have been difficult for cells to absorb. New encapsulated substances (nanoemusions)

are a new method for delivering oil-soluble substances to mammalian cell cultures. These transparent, phospholipid-stabilized nanoemulsions have high bioavailability, improving cell growth and vitality and allowing for toxicity studies of oil-soluble drugs in cell cultures [21].

5.7 Nanoemulsion as Antimicrobial Preparation

Theoretically, there are a number of methods of producing nanoemulsions, so they are able to function as antimicrobial carriers and enhance the safety and quality of food products. Unlike antibiotics, which have a particular antibacterial activity, nanoemulsions have a nonspecific antimicrobial action, which permits broad-range activity while reducing the potential for resistance development. Because of these advantages, nanoemulsions are a suitable choice for treating wounds and decontaminating surfaces [45].

5.8 Nanoemulsion to Improve the Per-Oral Delivery of Poorly Soluble Drugs

Nanoemulsion has wide applications in improving the solubility of poorly soluble drugs. BCS class II and IV drugs, which are poorly soluble in water, face challenges in conventional dosage forms. Nanoemulsions offer a solution for improved solubility and therapeutic efficacy [46].

5.9 Nanoemulsion in Transdermal Drug Delivery System

Nanoemulsion technology has been widely utilized as a transdermal drug delivery system. Nanomaterials' small size allows them to connect more substances due to their large surface area and easy transport, and their surface drainage allows them to accumulate at skin level [47].

5.10 Nanoemulsion in Targeted Drug Delivery System

As a targeted drug delivery system, nanoemulsion has been widely utilized in cancer therapy. As a result of their effective solubilization, biocompatibility, high stability, and capacity to aggregate in diseased areas with faulty vasculatures, overcoming anatomical and physiological barriers, nanoemulsions are being increasingly employed in cancer diagnostics, imaging, and therapy [48].

6. CONCLUSION

This study explores various nanoemulsion formulating techniques, preparation, characterization, release studies, and kinetic modeling. This article discusses the best techniques to produce nanoemulsions, which are: solvent displacement method, high-pressure homogenization, phase inversion by temperature, ultrasonication, microfluidization, and self-emulsifying nanoemulsion. All nanoemulsion formulations are generally considered effective, safe, and have improved bioavailability. Nanoemulsions offer advantages in drug delivery, masking oily liquids' unpleasant taste, and protecting drugs from hydrolysis and oxidation, making them popular for targeted anticancer, photosensitivity, and therapeutic agents. Moreover, various applications of nanoemulsion are also being discussed, including the use of nanoemulsion in drug delivery systems like oral, topical, intravenous, intramuscular, intranasal, pulmonary, and ocular. Nanoemulsion also has broad applications in cosmetics, cell culture technology, and antimicrobial preparation to improve the per-oral delivery of poorly soluble drugs in transdermal drug delivery systems.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We would like to extend our sincere gratitude to the Deanship of the Faculty of Pharmaceutical Sciences at Lahore University of Biological & Applied Sciences (UBAS) for providing the essential resources and support that made this research possible. Their commitment to fostering academic excellence has been invaluable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Borthakur P, et al., Nanoemulsion: Preparation and its application in food industry, in Emulsions. 2016;153-191.
2. Goindi S, et al., Nanoemulsions: an emerging technology in the food industry, in Emulsions. 2016;651-688.

3. Bhatt P, Madhav S. A detailed review on nanoemulsion drug delivery system. International Journal of Pharmaceutical sciences and research. 2011;2(10):2482.
4. Yukuyama M, et al., Nanoemulsion: process selection and application in cosmetics–a review. International journal of cosmetic science. 2016;38(1):13-24.
5. Pratap-Singh A, et al., Optimal ultrasonication process time remains constant for a specific nanoemulsion size reduction system. Scientific Reports. 2021; 11(1):9241.
6. Singh Y, et al., Nanoemulsion: Concepts, development and applications in drug delivery. Journal of controlled release. 2017;252:28-49.
7. Kumar M, et al., Techniques for formulation of nanoemulsion drug delivery system: a review. Preventive nutrition and food science. 2019;24(3):225.
8. Jafari SM, He Y, Bhandari B, Production of sub-micron emulsions by ultrasound and microfluidization techniques. Journal of food engineering. 2007;82(4):478-488.
9. Ganesan P, et al., Microfluidization trends in the development of nanodelivery systems and applications in chronic disease treatments. International journal of nanomedicine. 2018;6109-6121.
10. Vuillemard JC, Recent advances in the large-scale production of lipid vesicles for use in food products: microfluidization. Journal of microencapsulation. 1991;8(4): 547-562.
11. Chuesiang P, et al., Optimization of cinnamon oil nanoemulsions using phase inversion temperature method: Impact of oil phase composition and surfactant concentration. Journal of Colloid and Interface Science. 2018;514:208-216.
12. Ee SL, et al., Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. Chemical Engineering Journal. 2008;140 (1-3):626-631.
13. Jasmina H, et al. Preparation of nanoemulsions by high-energy and lowenergy emulsification methods. in CMBEBIH 2017: Proceedings of the International Conference on Medical and Biological Engineering; 2017.
14. Pathak C, Vaidya FU, Pandey SM. Mechanism for development of nanobased drug delivery system. Applications of targeted nano drugs and delivery systems. 2019;35-67.
15. Deng S, et al., Polymeric nanocapsules as nanotechnological alternative for drug delivery system: current status, challenges and opportunities. Nanomaterials. 2020; 10(5):847.
16. Pulingam T, et al., Exploring various techniques for the chemical and biological synthesis of polymeric nanoparticles. Nanomaterials. 2022;12(3):576.
17. Uppulurj KB, Self nano emulsifying drug delivery systems for oral delivery of hydrophobic drugs. Biomedical and pharmacology journal. 2015;6(2):355-362.
18. Dalal L, Allaf AW, El-Zein H. Formulation and in vitro evaluation of self-nanoemulsifying liquisolid tablets of furosemide. Scientific Reports. 2021;11 (1):1315.
19. Van Staden D, Du Plessis J, Viljoen J. Development of topical/transdermal self-emulsifying drug delivery systems, not as simple as expected. Scientia pharmaceutica. 2020;88(2):17.
20. Krahn CL. et al., Isoflurane-loaded nanoemulsion prepared by high-pressure homogenization: investigation of stability and dose reduction in general anesthesia. Journal of Biomedical Nanotechnology. 2012;8(5):849-858.
21. Ahmad N. et al., Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC advances. 2019; 9(35):20192-20206.
22. Firoozy M, Anarjan N. Preparation of maltodextrin stabilized α-tocopherol nanoemulsions using solvent-displacement technique. Food Science and Technology International. 2019;25(5):404-413.
23. Baloch J, et al., Self-nanoemulsifying drug delivery system (SNEDDS) for improved oral bioavailability of chlorpromazine: in vitro and in vivo evaluation. Medicina. 2019;55(5):210.
24. Silva HD, MÂ. Cerqueira, AA. Vicente, Nanoemulsions for food applications: development and characterization. Food and bioprocess technology. 2012;5:854-867.
25. Pathan I, Mangle M, Bairagi S. Design and characterization of nanoemulsion for transdermal delivery of meloxicam. Analytical chemistry letters. 2016;6(3):286-295.
26. Ho TM, Abik F, Mikkonen KS. An overview of nanoemulsion characterization via atomic force microscopy. Critical Reviews

in Food Science and Nutrition. 2022; 62(18):4908-4928.
27. Martins JT, et al., Protein-based structures for food applications: From macro to nanoscale. Frontiers in sustainable food systems. 2018;2:77.
28. Gurpreet K, Singh S. Review of nanoemulsion formulation and characterization techniques. Indian Journal of Pharmaceutical Sciences. 2018;80(5).
29. Mohammed NK, Muhialdin BJ, Meor Hussin AS. Characterization of nanoemulsion of Nigella sativa oil and its application in ice cream. Food science & nutrition. 2020;8(6):2608-2618.
30. Anarjan N, Tan CP. Effects of selected polysorbate and sucrose ester emulsifiers on the physicochemical properties of astaxanthin nanodispersions. Molecules. 2013;18(1):768-777.
31. Patravale V, Date AA. Kulkarni R. Nanosuspensions: a promising drug delivery strategy. Journal of pharmacy and pharmacology. 2004;56(7):827-840.
32. Alam A, et al., Babchi Oil-Based Nanoemulsion Hydrogel for the Management of Psoriasis: A Novel Energy Economic Approach Employing Biosurfactants. Gels. 2022;8(12):761.
33. Surh J, Decker EA, McClements DJ. Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. Food Hydrocolloids. 2006;20(5):607-618.
34. Alhamdany AT, AM. Saeed, Alaayedi M. Nanoemulsion and solid nanoemulsion for improving oral delivery of a breast cancer drug: Formulation, evaluation, and a comparison study. Saudi Pharmaceutical Journal. 2021;29(11):1278-1288.
35. Malik MR. et al., Formulation and characterization of chitosan-decorated multiple nanoemulsion for topical delivery in vitro and ex vivo. Molecules. 2022;27 (10):3183.
36. Vior MCG. et al., A comparative study of a novel lipophilic phthalocyanine incorporated into nanoemulsion formulations: Photophysics, size, solubility and thermodynamic stability. Dyes and Pigments. 2011;91(2):208-214.
37. Alhreez M, Xiao X, Wen D. Kinetic study of controlled asphaltene inhibitor release from nanoemulsions. Langmuir. 2019;35(33): 10795-10807.
38. Miastkowska M. et al., The kinetic study of isotretinoin release from nanoemulsion. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2016;510:63-68.
39. Sari T, et al., Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocolloids. 2015;43:540-546.
40. Ekenna IC, Abali SO. Comparison of the Use of Kinetic Model Plots and DD Solver Software to Evaluate the Drug Release from Griseofulvin Tablets. Journal of Drug Delivery and Therapeutics. 2022;12(2-S):5-13.
41. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. 2001;13(2):123-133.
42. Dash S. et al., Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67(3):217-223.
43. Gupta A. et al., Nanoemulsions: formation, properties and applications. Soft matter. 2016; 12(11):2826-2841.
44. Pathak K, Pattnaik S, Swain K. Application of nanoemulsions in drug delivery, in Nanoemulsions. 2018;415-433.
45. Shams N, Sahari MA. Nanoemulsions: preparation, structure, functional properties and their antimicrobial effects; 2016.
46. Thakur A, Walia MK, Kumar S. Nanoemulsion in enhancement of bioavailability of poorly soluble drugs: a review. Pharmacophore. 2013;4(1):15-25.
47. Wu Y, et al., The application of nanoemulsion in dermatology: an overview. Journal of drug targeting. 2013; 21(4):321-327.
48. Mahato R. Nanoemulsion as targeted drug delivery system for cancer therapeutics. Journal of pharmaceutical sciences and pharmacology. 2017;3(2):83-97.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113039